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**The genetics of pod-filling in peanut under water-limiting conditions**

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## *Abstract*

Pod-filling, an important yield-determining stage is strongly influenced by water stress. This is particularly true for peanut (*Arachis hypogaea*), wherein pods are developed underground and are directly affected by the water condition. Pod-filling in peanut has a significant genetic component as well, since genotypes are considerably varied in their pod-fill (PF) and seed-fill (SF) potential. The goals of this research were to:
(1) Examine the effects of genotype, irrigation, and genotype X irrigation on PF and SF.
(2) Detect global changes in mRNA and metabolites levels that accompany PF and SF.
(3) Explore the response of the duplicate peanut pod transcriptome to drought stress.
(4) Study how entire duplicated PF regulatory processes are networked within a polyploid organism.
(5) Discover locus-specific SNP markers and map pod quality traits under different environments.
The research included genotypes and segregating populations from Israel and US that are varied in PF, SF and their tolerance to water deficit. Initially, an extensive field trial was conducted to investigate the effects of genotype, irrigation, and genotype X irrigation on PF and SF. Significant irrigation and genotypic effect was observed for the two main PF related traits, "seed ratio" and "dead-end ratio", demonstrating that reduction in irrigation directly influences the developing pods as a result of low water potential. Although the Irrigation × Genotype interaction was not statistically significant, one genotype (line 53) was found to be more sensitive to low irrigation treatments. Two RNAseq studies were simultaneously conducted in IL and the USA to characterize expression changes that accompany shell ("source") and seed ("sink") biogenesis in peanut. Both studies showed that SF and PF processes are very dynamic and undergo very rapid change in the accumulation of RNA, nutrients, and oil. Some genotypes differ in transcript accumulation rates, which can explain their difference in SF and PF potential; like cv Hanoch that was found to be more enriched than line 53 in processes involving the generation of metabolites and energy at the beginning of seed development. Interestingly, an opposite situation was found in pericarp development, wherein rapid cell wall maturation processes were up-regulated in line 53. Although no significant effect was found for the irrigation level on seed transcriptome in general, and particularly on subgenomic assignment (that was found almost comparable to a 1:1 for A- and B- subgenomes), more specific homoeologous expression changes associated with particular biosynthesis pathways were found. For example, some significant A- and B- biases were observed in particular parts of the oil related gene expression network and several candidate genes with potential influence on oil content and SF were further examined. Substation achievement of the current program was the development and application of new SNP detection and mapping methods for peanut. Two major efforts on this direction were performed. In IL, a GBS approach was developed to map pod quality traits on Hanoch X 53 F2/F3 generations. Although the GBS approach was found to be less effective for our genetic system, it still succeeded to find significant mapping locations for several traits like testa color (linkage A10), number of seeds/pods (A5) and pod wart resistance (B7). In the USA, a SNP array was developed and applied for peanut, which is based on whole genome re-sequencing of 20 genotypes. This chip was used to map pod quality related traits in a Tifrunner x NC3033 RIL population. It was phenotyped for three years, including a new x-ray method to phenotype seed-fill and seed density. The total map size was 1229.7 cM with 1320 markers assigned. Based on this linkage map, 21 QTLs were identified for the traits 16/64 weight, kernel percentage, seed and pod weight, double pod and pod area. Collectively, this research serves as the first fundamental effort in peanut for understanding the PF and SF components, as a whole, and as influenced by the irrigation level. Results of the proposed study will also generate information and materials that will benefit peanut breeding by facilitating selection for reduced linkage drag during introgression of disease resistance traits into elite cultivars.

## Summary Sheet

## Publication Summary

| PubType | IS only | Joint | US only |
|---|---|---|---|
| Book Chapter | 0 | 1 | 0 |
| Reviewed | 1 | 2 | 0 |

## Training Summary

| Trainee Type | Last Name | First Name | Institution | Country |
|---|---|---|---|---|
| Postdoctoral Fellow | Gupta | Kapil | ARO | Israel |
| Ph.D. Student | Chavarro | Carolina | UGA | USA |

***Contribution of the collaboration***: This project is based upon high level of communication throughout the project between PIs Hovav, Ozias-Akins, and Jackson and among PIs and graduate students, Carolina Chavarro (BARD-funded) and Josh Clevenger (funded through other projects, but involved in transcriptome and SNP analysis for this and other projects). Also, Dr. Kapil Gupta (BARD-funded) from Hovav lab was highly communicated with Co-Pi Ozias-Akins lab. PI Hovav contribution was mainly in the field trial experimental design and analysis and transcriptomics data analysis and mining. Co-PI Ozias has directed the discovery of locus-specific SNPs markers and trait mapping methods that were used in both countries. The collaboration included very intense information exchange with e-mails between the two lab members on almost daily basis and at least five actual meetings between the PIs. This is reflected by high collaborative efforts like, for example, four Israeli lines that were re-sequenced and were used as part of the above mentioned SNP-array design. The close communication between the labs is also reflected in the joined publications listed below.

## *Achievements*

Below, the scientific and agricultural achievements are described with accordance to the objectives.

**Objective 1: Examine the effects of genotype, irrigation, and genotype X irrigation on PF and SF.** A detailed analysis of SF, PF and other pod quality traits was carried out in field conditions with 4 irrigations (100%, 120%, 80% and 60%) and 4 replications. The field test was performed in Western Negev, IL. Four Israeli genotypes were used, including 2 with presumably good PF potential (Hanoch, A80) and 2 genotypes with incomplete PF (53, B18). Line 53 is the local name for PI338338, a Peruvian-type peanut. The "commercial" (100%) irrigation was determined by 4 lysimeter devices. Average seed percentage (SP), "dead end" ratio (DE) and other important pod related traits were measured. Hanoch and A80 had the highest SP, with no respect to the level of irrigation. Line 53 had the lowest SP as speculated. Significant effect for irrigation level was found on SP and DE, which were gradually affected from decreasing irrigation levels, but only at 60% was significantly lower. Although the Irrigation×Genotype interaction was not statistically significant, line 53 was found to be very sensitive for the irrigation reduction, in which the effect in 80% is already significant. *This trial served as a fundamental study for understanding the pod-filling components that are influenced by the irrigation level in different genotypes and was a prerequisite for some parts of the following investigations*. *Also, new irrigation guidelines for farmers in that area were made based in part of the results of this trial (see the attached publication).*

**Objective 2: Detect global changes in mRNA levels that accompany pod and seed development**. Expression changes that accompany pod shell ("source") and seed ("sink") biogenesis in peanut were characterized. Two RNAseq experiments were performed. In the USA, Tifrunner and NC 3033, two parental lines contrasting for PF and SF were grown in controlled conditions. Four stages (R4-R7) and three tissues (embryos, seed coats and pericarps) were analyzed. Comparison of the differentially expressed (DE) genes in the interaction of genotype by all tissues and developmental stages revealed *that between Tifrunner and NC 3033 there is not a big gene expression difference in pericarp tissue, which protects and serves as a channel for essential nutrients and assimilates, but there is substantial difference at seed coat level which surrounds the developing embryo and helps to nourish and protect the embryo from the external environment*. Regarding the interaction Genotype x Tissue there was a large difference between the DE genes in R4-5 vs R6 and R7, indicating that R4-5 *is a decisive stage for the genotypes in the peanut pod and seed development where the pod elongation and beginning of seed formation occurs*. R4-5 correspond to the stage where the seed storage protein synthesis is highly active, enriched in functional genes that belong to metabolic pathways such as carbohydrate, amino acid, lipid, and energy. In Israel, two genotypes, Hanoch and 53 were used. The

experiment was an integrated part of the irrigation test described above. Pods from the same developmental stages (R4-7) were harvested but only pericarps and seeds were analyzed. Transcriptome data were generated by RNA-Seq and explored with respect to genic and subgenomic patterns of expression. With agreement to the US trial, the SF process was found very dynamic, in which many transcriptomic changes were observed in both genotypes during development. Yet, higher expression and more enriched processes involving the generation of energy and primary metabolites were observed for the genotype with the better PF (Hanoch). *A new peanut dataset of 584 oil-related genes was assembled and analyzed*, resulting in several lipid metabolic processes highly expressed in Hanoch, including oil storage and FA biosynthesis. A parallel analysis was performed on developing pericarps. The dynamics of pericarp related gene expression was fairly different from what was found in the seed. While in seeds the big transition occurred very early in seed development in line 53, in the shell the greatest change was between the R5-R6. Moreover, specific changes for cell wall biosynthesis and for cellulose biosynthesis were found to be more enriched in 53 than Hanoch at the early developmental stages. In particular, several CesA genes, related to secondary cell wall biosynthesis (CesA4, CesA7, CesA8) had higher expression at R5 stage in line 53 compared with Hanoch. This is distinctive for *early synthesis of secondary cell well and early maturation of line 53 pericarps*. General metabolite analysis of the pericarp was done for the same samples, and significantly higher levels of cellulose, hemicellulose, calcium and ash were found in line 53 compared to Hanoch during development. *Collectively, the differences in these metabolites, together with the higher levels of CesA4,7,8 expression may explain the early maturation of the pod shell in line 53, which can lead to decrease in water permeability to the developing seeds, and eventually interference to the proper seed and pod filling processes.*

**Objective 3: The response of the duplicate peanut pod transcriptome to drought stress.** In another RNAseq trial, performed in IL, developing seeds and pods were collected from line 53 growing in 100% and 60% irrigation levels. No significant effect of the irrigation on seed and pericarp trascriptome was found in general, and particularly on sub-genomic assignment. In both tissues, the total number of DE genes between irrigation treatments was low, with no particular enriched biological processes. The assignment of the DE genes to A- or B- genome was close to 50%. *Therefore, the direct irrigation level does not have significance effect on the duplicate genome*.

**Objective 4: Study how entire duplicated PF processes are networked within a single polyploid.** Global transcript abundance of the duplicated genome of peanut was investigated, and was found all through the study corresponding with almost comparable to a 1:1 ratio for the A- vs B- subgenomes. Yet, further investigation of more specific homoeologous gene expression changes associated with particular biosynthesis pathways, like the oil biosynthesis pathway, found significant results. *Biases*

*were observed in particular parts of the pathway with possible biological meaning, which may explain the genotypic variation in oil biosynthesis and PF* (see publication attached for more details).

**Objective 5: Discover locus-specific SNPs markers and map pod quality traits under different environments.** For SNP detection, 20 genotypes of interest, 5 of direct relevance to the BARD project, were selected for whole genome re-sequencing. The high quality reads were mapped over the two diploid genomes. In-house SWEEP program was used for the SNP calling. After the selection of the SNPs fitting the Affymetrix design 58,233 SNPs were selected in a *chip for genotyping samples of interest in peanut not only tetraploid but also diploid species*. Two populations were inspected for pod quality traits. In Israel, F2-F3 generations of the cross Hanoch X 53 were analyzed by GBS approach. Seed percentage and other important pod related traits were measured. Relatively large deviation was found in both generations towards extra lower seed percentage values. Broad and narrow sense heritability estimates were found to be relatively high (0.8 and 0.68, respectively). That indicates for a large genetic component for the seed ratio trait. The process of both SNP detection and SNP marker application in the progenies was conducted by GBS approach on 94 F2 progenies. Association mapping was conducted by GLM function of the TASSEL software, revealing for significant result for the testa color trait, which was mapped to the linkage group A10 with $R^2$ of 0.48 for the best SNP. QTL were found in this GBS analysis for the number of seeds per pod in 2013 and 2014 (linkage group A5; $R^2$ =0.28 ) and pod wart resistance (linkage group B7 ; $R^2$ = 0.18). No significant QTL was found for the seed ratio trait. In the USA, The RIL population derived from Tifrunner x NC3033 was phenotyped for pod and seed traits in Tifton in 2013, 2014 and 2015. Pod and seed traits were obtained to determine 16/64 weight (weight of the seeds with size of interest) and percentage, seeds per pod, and kernel weight and percentage, seed density and more. Based on the Affymetrix array, we obtained 2,233 polymorphic SNPs for this RIL population. After filtering 1,998 SNPs and 100 SSRs were selected for genetic mapping. The linkage map order was defined by comparison with the pseudomolecule order position of the two diploid genomes. Twenty-one QTLs were identified in the linkage groups A05, A10, A07_B07, B04a, B06, B07a and B10a for the traits 16/64 weight 2013 and percentage 2013-2014, kernel percentage 2015, seed and pod weight 2014 - 2015, double pods 2014 and pod area 2014. *Nine and eight QTLs were found in the linkage groups A10 and A07_B07 respectively, close to the markers AX147226969 and the flanking region between AX147226917 - AX147226852*. These QTLs were detected for seed weight 2014-2015 and pod weight 2014-2015, all of them determined by the parental NC3033. Tifrunner contributed for 6 QTLs on the linkage groups for kernel % 2015, pod weight 2014, double pods 2014 and pod area 2014. NC3033 contributed most of the QTLs with the phenotypic variation percentage ranging from 1.3% for 16/64 % 2013 to 29.4% for 16/64 weight 2013.

## Publications for Project IS-4540-12

| Status | Type | Authors | Title | Journal | Vol:pg Year | Coun |
|---|---|---|---|---|---|---|
| Published | Reviewed | *Gupta, K., Buchshtab, O., Hovav, R.* | The effects of irrigation level and genotype on pod-filling related traits in peanut (Arachis hypogaea) | ***J. Agri. Sci.*** | 7(1) : 2014 | IS only |
| Accepted | Reviewed | *Clevenger, J., Chu, Y., Chavarro, C., Agarwal, G., Bertioli, D.J., Leal-Bertioli, S.C.M., Pandey, M.K., Vaughn, J., Abernathy, B., Barkley, N., Hovav, Ran., Burow, M., Nayak, S.N., Chitikineni, A., Ozias-Akins* | Genome-wide SNP Genotyping Resolves Signatures of Selection and Tetrasomic Recombination in Peanut | ***Molecular plant*** | : 2016 | Joint |
| Published | Reviewed | *Gupta, K., Kayam, G., Faigenboim-Doron, A., Clevenger, J., Ozias-Akins, P., & Hovav, R* | Gene expression profiling during seed-filling process in peanut with emphasis on oil biosynthesis networks | ***Plant Science*** | 248 : 116-127 2016 | Joint |
| Published | Book Chapter | *Chu, Y., J. Clevenger, R. Hovav, J. Wang, B. Scheffler, S.A. Jackson, P. Ozias-Akins* | Application of Genomic, Transcriptomic, and Metabolomic Technologies in Arachis Species | | : 2016 | Joint |

# 2. Appendix

## A. List of abstract of presentation in conferences:

Chavarro C., Abernathy, B., Bertioli, D., Jackson, S., Holbrook, C.C., Clevenger, J., **Ozias-Akins, P**. 2015. Identification of SNPs for Arachis hypogaea L. Genotypes using WGS Based on the Two Diploid Reference Genomes. APRES 47:159.

Chavarro C., **Ozias-Akins, P.**, Jackson, S.A., Abernathy, B. 2015. Differential expression during seed and pod biogenesis through RNA-Seq analysis. AAGB 2015, 5-8 Nov, Brisbane, Australia

Clevenger, J., Chu. Y., Bertioli, D., Bertioli, S., Scheffler, B., Froenicke, L., **Hovav, R.**, Conner, J., Abernathy, B., Jackson, S., Holbrook, C., **Ozias-Akins**. P. 2014. The *Arachis* transcriptome. 7th Intl. Conf. Adv. Arachis through Genomics & Biotechnology, 10-14 Nov., Savannah, GA

Clevenger, J., Chavarro, C., Pearl, S., Jackson, S., **Ozias-Akins**, **P.** 2014. Prospects for a SNP chip in cultivated peanut (*Arachis hypogaea*) utilizing the leaf transcriptome. 7th Intl. Conf. Adv. Arachis through Genomics & Biotechnology, 10-14 Nov., Savannah, GA.

Clevenger, J., Chavarro, C., Abernathy, B., Agarwal, G., Pandey, M., Bertioli, D., Jackson, S., Varshney, R., **Ozias-Akins, P**. 2015. Identification of large-scale SNPs for the development of a 60 K SNP array in groundnut. AAGB 2015, 5-8 Nov, Brisbane, Australia

Gupta, K., Kayam, G., Doron, A., Clevenger**,** J.P.**, Ozias-Akins, P.**, **Hovav, R**. 2015. Transcriptome profiling of developing peanut seed with a focus on oil related expression networks. APRES 47:50.

**Ozias-Akins P**, Clevenger, J., Chu, Y., Guimaraes, L, Maia, T., Huang, W., Duke, M., Scheffler, B., Cannon, S. 2015. Gene expression profiling in cultivated peanut: Putative gene functions and candidate gene discovery. AAGB 2015, 5-8 Nov, Brisbane, Australia

## C. Papers in preparation, in submission or under review:

Pandey, M., G. Agarwal, S. Kale, J. Clevenger, S. Nayak, M. Sriswathi1, A. Chitikineni, C. Chavarro, X. Chen, H.D. Upadhyaya, M.K Vishwakarma, S. Leal-Bertioli, X. Liang, D.J. Bertioli., B. Guo, S.A. Jackson, **P. Ozias-Akins**, R.K. Varshney. 2016. A powerful and high density genotyping tool for peanut genetics and breeding: development and evaluation of 58K Axiom_Arachis SNP array. Scientific Reports. accepted for publication.

Gupta, K., Patil, A., Doron A.**, Hovav, R.** Global gene expression analysis of peanut developing pericarps revealed an important role for cell wall biogenesis gene network in early pod maturation. In submission to a special issue of Frontiers in Plant Science on: "Advances in legume research".

Other manuscripts in preparation include results from transcriptional profiling and genetic mapping of pod and seed biogenesis described above.